USING FLUORESCENCE IMAGERY AND MICROBES FOR ORDNANCE AND MINE DETECTION

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Introduction

The cost to remediate sites that contain landmines, unexploded ordnance (UXO), and contamination from high-explosive (HE) materials is staggering. Because of the ever-increasing demand for land resources, this problem will continue to grow in importance, both within and outside of the United States. Landmines and other forms of UXO cover vast areas in countries such as Bosnia, Angola, and Cambodia. As such, their impact is immense in terms of civilian casualties and the loss of productive farming and grazing land. Within the United States, injuries caused by these devices are rare, but their impact on restricting land use is still high.

Cost-effective methods must be developed to survey and remediate installation impact areas, training zones, and munitions disposal sites before they are considered for new usage (under legislation such as Base Realignment and Closure (BRAC)). If the locations of the landmines, UXO, and contamination are accurately mapped, remediation can be done safely and at a substantially reduced cost.

A new approach with exciting implications is to combine fluorescent biosensors and Laser-Induced Fluorescence Imaging (LIFI). This technique was originally developed to locate landmines, but can be used on all types of explosive devices. Usage requires only that a minute amount of explosive leak from the device. The detector is a strain of genetically engineered bacteria that produces a fluorescent protein when in contact with trinitrotoluene (TNT). This innovative technique is a potentially powerful tool in detecting landmines, UXO, and HE contamination. Because both bacteria delivery and detection can be accomplished from airborne platforms, this technique is far safer than ground-based systems and can cover large areas in short periods of time.

Background

The actual detection system is composed of two parts. The first part is the microorganism *Pseudomonas putida*, a naturally occurring, innocuous soil bacteria that has a protein that recognizes TNT and expresses (turns on) a gene in response. This bacterial gene has been genetically fused to a second gene called *green fluorescent protein* (*gfp*), by scientists at the Oak Ridge National Laboratory (ORNL). The *gfp* gene, found naturally in the jellyfish *Aequoera victoria*, produces a protein that has a strong fluorescence in the green region (510nm) of the visible light spectrum. When in the presence of TNT, these bacteria are very easy to detect because of the *gfp* fluorescence. The bacteria can be grown and harvested in high numbers using standard growth media. When applied in the field, the bacteria will search for organic molecules that they can use as food. When the bacteria come into contact with TNT, they will attempt to digest it with their enzymes, but will instead produce the fluorescent protein.

The second part of the system involves detecting the fluorescent microbe. The detection instrument used is the LIFI system developed by Bechtel Nevada Special Technologies Laboratory (STL). The LIFI system was initially developed to detect surface uranium contamination. The LIFI instrument is a 35-pound backpack portable system that includes a laser power supply and onboard computer. LIFI uses an eye-safe, pulsed ultraviolet (355nm) laser as the excitation source. An intensified charge-coupled device camera captures ambient background and fluorescence imagery. Onboard hardware and software controls the laser, captures imagery, eliminates the background, and produces a real-time display showing fluorescence intensity. All imagery can be saved to a hard disk for processing and further analysis.

Initial Test

This combined technology was demonstrated at the National Explosives Waste Technology and Evaluation Center (NEWTEC) in Edgefield, SC. The site was proposed as a realistic minefield, with surrogate landmines containing TNT filler planted at locations unknown to the scientific team. The mines were buried approximately 3 months before the demonstration took place to allow the mines enough time to leak explosives into the soil. The resulting conditions were similar to actual abandoned minefields in temperate woodland climates. All targets were buried at least 4 inches beneath the surface with no evidence of soil disturbance.

Using a tractor equipped with an agricultural sprayer and a modified agricultural spraying boom, the bacteria were applied to the quarter-acre test plot. The application solution was prepared by loading a quantity of the bacteria into a 150-gallon reservoir, then adding site well water (nonchlorinated) to bring the total volume up to the desired level. Four-and-a-half liters of bacterial solution, containing approximately 10^{13} total bacteria, was added to approximately 75-80 gallons of water. The test plot was first sprayed with water to saturate the soil prior to applying the bacterial solution.

Both a gantry system and mobile platform were used to position the LIFI sensing head in a nadir orientation over the test area. Typical working distances were 4-8 meters above the surface. The LIFI system located four of the five blind targets within a distance of 2 meters. The remaining target was identified about 3 meters from its actual position. Two false positives were also identified, one of which involved TNT migration caused by heavy rainfall. The second false positive was not near any buried mine, however, soil sampling showed TNT on the surface of this location.
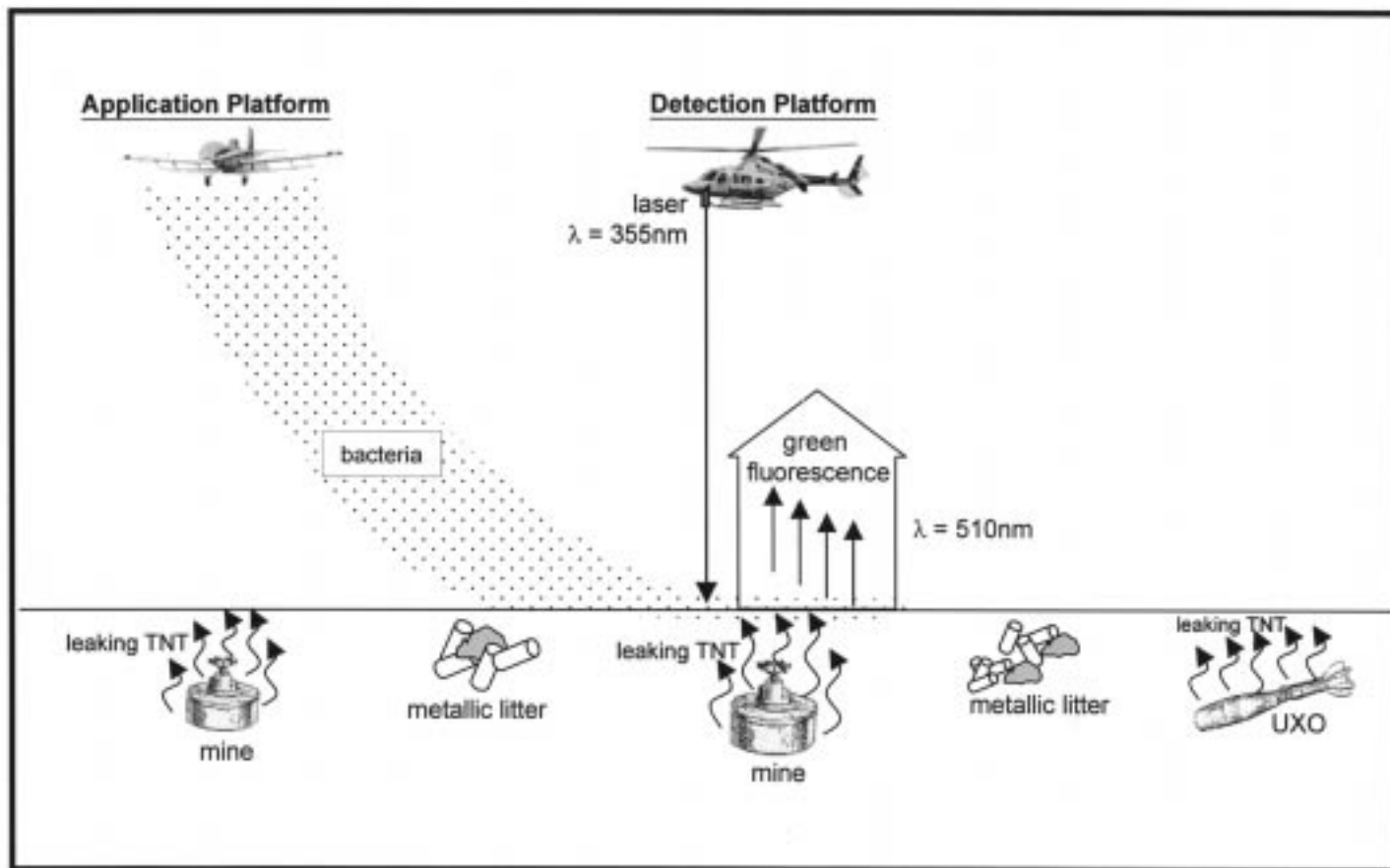
Figure 1.
Proposed operational system

Full System Development

Based upon these encouraging results, a multiagency team (ORNL, STL, U.S. Army Engineer Research and Development Center, and Los Alamos National Laboratory) is pursuing funding to further test and eventually develop an operational mine detection system (Figure 1). A fully operational system would consist of bacteria creation and airborne delivery components, airborne laser-induced fluorescence and imaging components, and software to perform signature matching and geographic information system (GIS) product generation. A breakout of the airborne detection and ground-based components is shown in Figure 2.

Of the three components, bacteria growth and delivery is the most mature. Techniques for growing large amounts of bacteria are well known, as are methods of aircraft delivery via crop dusters. However, research is still needed on the bacteria to amplify the fluorescence signal, on refining the position of the wavelength maximum, and for adapting more bacterial strains to detect other explosive materials, such as RDX (hexahydro-trinitro-triazine).

Scaling the current LIFI system to an airborne platform is feasible. STL has built two airborne fluorosensor systems, one of which is similar to the technology used in the NEWTEC experiment. The system could be fielded in Army helicopters without airframe modification. Increased laser power would be required to illuminate larger areas from higher altitudes, and the imaging hardware would require improvement to detect lower fluorescence levels. Hardware capable of meeting these requirements should be commercially available within the proposed 4-year development cycle.

The software system would produce a georegistered mosaic by combining the aircraft's Differential Global Positioning System (DGPS) and inertial measurement unit (IMU) with the fluorescence imagery. The imagery would then be compared to emission spectra from a spectral database to separate out background features (i.e., soils and vegetation) and identify "hot" regions. The final product would be multiband fluorescence images and overlays that are compatible with existing GIS and image processing systems.

The goal for a fully functional system is to cover approximately 25 acres per hour. If this technology succeeds, it will represent a breakthrough in coverage capability.

Concerns

As with any new technology, certain drawbacks, limitations, and assumptions must be addressed. The public perception of using genetically modified bacteria is an obvious concern. Hopefully, these concerns can easily be dispelled. The Environmental Protection Agency has reviewed the bacteria construction and has approved limited releases. Functionally, the genetically altered bacteria have no competitive advantage over nonengineered bacteria. The only major difference is that the modified bacteria "turn on" the *gfp* when they come into contact with the target substance (in this case TNT).

As with any detection instrument, the proposed system should be tested under a variety of different environmental and meteorological conditions. Questions about TNT transport and chemical interaction with the soil must also be addressed. Other researchers in this field have studied many of these parameters and their results should
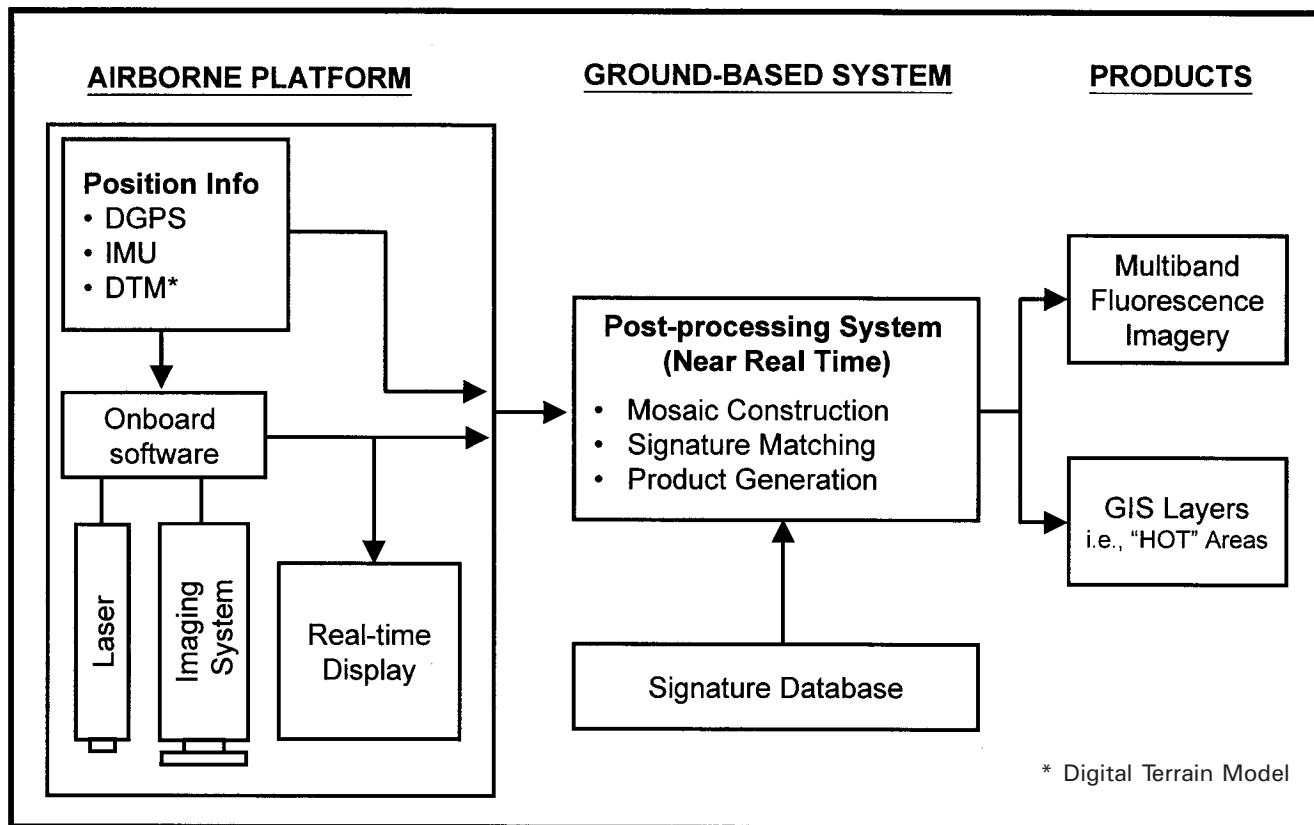
Figure 2.

AIRBORNE PLATFORM   GROUND-BASED SYSTEM   PRODUCTS

Position Info
- DGPS
- IMU
- DTM*

Onboard software

Laser

Imaging System

Real-time Display

Post-processing System (Near Real Time)
- Mosaic Construction
- Signature Matching
- Product Generation

Signature Database

Multiband Fluorescence Imagery

GIS Layers
i.e., "HOT" Areas

* Digital Terrain Model

Figure 2.
Block diagram of airborne and ground-based components

provide insight into the full capability of this system.

A final concern is that the landmines or UXO would need a minimum amount of time to allow the TNT to reach the ground surface. This will probably limit the use of this technology in tactical situations. However, in most humanitarian and other civil applications, the landmines and UXO have been in place for several years and have been subject to considerable diurnal and seasonal effects.

Potential Payoff

Having a single technique work as a "silver bullet" for the landmine and UXO problem is unlikely. We would advocate the fusion of our proposed system with existing and future detection platforms. However, a new technology that can detect landmines and UXO over a wide range in a timely manner would be of substantial benefit. When considering both the worldwide humanitarian and monetary costs from land-mines and UXO, any system that can pro-vide even moderate safety and efficiency improvements would be a breakthrough.

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